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Antigen–Antibody Interactions and Monoclonal Antibodies

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The basic principles of antigen–antibody interaction are those of any bimolecular reaction. Moreover, the binding of antigen by antibody can, in general, be described by the same theories and studied by the same experimental approaches as the binding of a hormone by its receptor, of a substrate by enzyme, or of oxygen by hemoglobin. There are several major differences, however, between antigen–antibody interactions and these other situations. First, unlike most enzymes and many hormone-binding systems, antibodies do not irreversibly alter the antigen

they bind. Thus the reactions are, at least in principle, always reversible. Second, antibodies can be raised, by design of the investigator, with specificity for almost any substance known. In each case, one can find antibodies with affinities as high as and specificities as great as those of enzymes for their substrates and receptors for their hormones. The interaction of antibody with antigen can thus be taken as a prototype for interactions of macromolecules with ligands in general. In addition, these same features of reversibility and availability of a wide variety of specificities have made antibodies invaluable reagents for identifying, quantitating, and even purifying a growing number of substances of biological and medical importance.

One other feature of antibodies that, until recently, has proved to be a difficulty in studying and using them, compared to, say, enzymes, is their enormous heterogeneity. Even "purified" antibodies from an immune antiserum, all specific for the same substance and sharing the same overall immunoglobulin structure (see Chapter 9), will be a heterogeneous mixture of molecules of different subclass, different affinity, and different fine specificity and ability to discriminate among cross-reacting antigens. The recent advent of hybridoma monoclonal antibodies (1–3) (see below, pp. 347ff) has made available a source of homogeneous antibodies to almost anything to which antisera can be raised. Nevertheless, heterogeneous antisera are still in widespread use and even have advantages for certain purposes, such as precipitation reactions. Therefore it is critical to keep in mind throughout this chapter, and indeed much of the volume, that the principles derived for the interaction of one antibody with one antigen must be modified and extended to cover the case of heterogeneous components in the reaction.

In this chapter we examine the theoretical principles necessary for analyzing, in a quantitative manner, the interaction of antibody with antigen, and the experimental techniques that have been developed to study these interactions as well as to make use of antibodies as quantitative reagents. In addition, we explore the effects of antigen binding on the antibody molecule itself. Further, we discuss the derivation, use, and properties of monoclonal antibodies.

THERMODYNAMICS AND KINETICS

The Thermodynamics of Affinity

The basic thermodynamic principles of antigen–antibody interactions, as we indicated above, are the same as those for any reversible bimolecular binding reaction. We review these as they apply to this particular immunological reaction.

Chemical Equilibrium in Solution

For this purpose, let S = antibody binding sites, L = ligand (antigen) sites, and SL = the complex of the two. Then for the reaction

$$S + L \rightleftharpoons SL \tag{1}$$

the mass action law states

$$K_A = \frac{[SL]}{[S][L]} \tag{2}$$

where K_A = association constant (or affinity) and square brackets indicate molar concentration of the reactants enclosed. The import of this equation is that, for any given set of conditions such as temperature, pH, and salt concentration, the ratio of the concentration of the complex to the product of the concentrations of the reactants at equilibrium is always constant. Thus changing the concentration of either antibody or ligand will invariably change the concentration of the complex, provided neither reactant is limiting, that is, neither has already been saturated, and provided sufficient time is allowed to reach a new state of equilibrium. Moreover, since the concentrations of antibody and ligand appear in this equation in a completely symmetrical fashion, doubling either the antibody concentration or the antigen concentration results in a doubling of the concentration of the antigen–antibody complex, provided the other reactant is in sufficient excess. This proviso, an echo of the one just above, is inherent in the fact that [S] and [L] refer to the concentrations of free S and free L, respectively, in solution, not the total concentration, which would include that of the complex. Thus, if L if not in great excess, doubling [S] results in a decrease in [L] as some of it is consumed in the complex, so the net result is less than a doubling of [SL]. Similarly, halving the volume results in a doubling of the total concentration of both antibody and ligand. If the fraction of both reactants tied up in the complex is negligibly small (as might be the case for low affinity binding), the concentration of the complex quadruples. However, in most practical cases, the concentration of complex is a significant fraction of the total concentration of antigen or antibody or both, so the net result is an increase in the concentration of complex, but by a factor of less than four. The other important, perhaps obvious, but often forgotten, principle to be gleaned from this example is that since it is concentration, not amount, of each reactant that enters into the mass action law [Equation (2)], putting the same amount of antigen and antibody in a smaller volume will increase the amount of complex formed, and diluting them in a larger volume will greatly decrease the amount of complex formed. Moreover, these changes go approximately as the square of the volume, so volumes are critical in the design of an experiment.

The effect of increasing free ligand concentration [L], at constant total antibody concentration, on the concentration of complex, [SL], is illustrated in Fig. 1. The mass action law [Equation (2)] can be rewritten

$$[SL] = K_A[S][L] = K_A([S]_t - [SL])[L] \tag{3}$$

or

$$[SL] = \frac{K_A[S]_t[L]}{1 + K_A[L]} \tag{3'}$$

where $[S]_t$ = total antibody site concentration; that is, [S] + [SL]. Initially, when the complex [SL] is a negligible